

COMMENTARY

Resistance is skin-deep: innate immunity may help amphibians to survive a deadly fungus

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Innate immune defenses provide fast and efficient protection against most pathogens and parasites. Yet most immunology textbooks extensively deal with adaptive (i.e. acquired) immunity, while devoting little space to innate immunity. It is only quite recently that immunologists are becoming increasingly interested in this evolutionary more ancient type of defense (Hoffmann *et al.*, 1999; Janeway & Medzhitov, 2002). However, how important is innate immunity for protection against naturally occurring pathogens? Might it be even more relevant in non-mammalian species, such as cold-blooded animals, where adaptive immunity might be slow to develop? Could it be that innate defenses are the most relevant when it comes to resisting emerging infectious diseases? Ecological immunologists are starting to address these urgent questions.

The work by Woodhams *et al.* (2007) is an impressive example serving as a motivation for further studies that merge ecology and immunology. It addresses the problem of fungal infections of the skin of anuran species, in a context of worldwide decline of amphibian populations. Understanding of the impact of infectious diseases on wild populations is still in its infancy. Nevertheless, all indications are that infectious diseases can significantly influence dynamics of wild populations, especially in scenarios of ongoing environmental change, which may alter the balance of host–parasite interactions and may facilitate the spread of pathogens. In such a scenario, immunocompetence is crucial to the survival and reproduction of afflicted hosts. It is thus imperative to investigate how wild animals can defend themselves against both resident and invading pathogens. The coevolutionary interaction of hosts and pathogens may lead to a seemingly balanced situation of reciprocal adaptation (Thompson, 1994). However, changing environments and the invasion of locally ‘new’ pathogens may delicately alter this balance of forces, potentially culminating in the decline or even extinction of afflicted populations.

The information provided by Woodhams *et al.* (2007) significantly contributes to the understanding of how anuran species can defend fungal infections through antimicrobial peptides in their skin mucus layer. Mucus extracts from all four tested species were able to inhibit fungal growth, but to a different degree, such that the minimal inhibitory concentration equivalents per cm² skin of the diverse species tended to increase with survival rates of the species after experimental exposure to the fungus. However, because extracts from all tested species had inhibitory effects, additional factors of innate and/or adaptive immunity might contribute to the extreme differences in survival rates observed among the species. This conjecture is corroborated by the fact that although three of the four species were found to be highly susceptible after experimental infection, none of the species included in this study is known to be in population decline.

Species variation in the encounter rate with the pathogen in the wild might here be an important issue. Encounter probability might have resulted in diverse adaptations in addition to the repertoire of antimicrobial peptides. Tree-living frogs naturally might have a lower encounter rate with chytridiomycosis and therefore might be more susceptible to the infection. An association of inferior resistance with lower encounter probability was for example found in populations of a teleost fish, the three-spined stickleback *Gasterosteus aculeatus*, and related to differences in innate immunity (Kalbe & Kurtz, 2006; Scharsack *et al.*, 2007). Estimation of the encounter rate in the wild, its local and seasonal dynamics, is needed to understand how the skin fungus infection may affect wild anuran populations.

One of the main strengths of the approach chosen by Woodhams *et al.* (2007) is the experimental exposure of diverse host species to a naturally relevant pathogen in combination with immunological analyses in one of those species. By contrast, the majority of studies in the young

field of ecological immunology tends to make use of rather artificial immune elicitors and/or a limited set of immune assays (Schmid-Hempel, 2003; Adamo, 2004). Clearly, either way has proven successful, but we would wish to see more studies taking the rather laborious, but promising approach that includes both naturally occurring pathogens and relevant immune traits.

On a more cautionary note, however, Woodhams *et al.*'s study also has some limitations that await further advance in future work. The authors investigated offspring from a single sibship per species, but tend to conclude that resistance varies among different anuran species. Variation in resistance and immunity among sibships can be enormous, even when parents originate from the same habitat, as has for example been demonstrated in the three-spined stickleback (Rauch, Kalbe & Reusch, 2006; Wegner, Kalbe & Reusch, 2007). In Woodhams *et al.*'s study, it cannot be ruled out that family effects (within species) contribute to the observed differences in infection and immunity. An experimental setup with several families per species would be needed to substantiate the conclusion that differences in susceptibility are indeed species-specific. Moreover, for obtaining skin peptides, Woodhams *et al.* decided to use control (i.e. non-exposed) frogs, which can provide information only on constitutive defenses. However, innate defenses are often inducible and might even show specific priming upon prior contact with a pathogen (Kurtz, 2005). In future studies, it will thus be interesting to see whether prior exposure to the pathogen enhances protection through skin peptides. Likewise, blood cell counts were obtained from *Litoria chloris* frogs after exposure, but without knowledge of their ability to overcome the infection. Given that a considerable proportion of *L. chloris* showed no detectable infection at 108 days post-exposure in the survival experiment, it is quite likely that the group of exposed *L. chloris* could be sub-divided into susceptible versus resistant frogs. Any immunological differences between these groups would be highly informative as to the relevant defenses forestalling the establishment of an infection.

One of the most interesting conclusions from Woodhams *et al.*'s study is that innate rather than adaptive immunity seems to be causal for the defense against the skin fungus infection. It is well established that anurans are endowed with all components characterizing the vertebrate adaptive immune system, such as immunoglobulin, major histocompatibility complex (MHC), B- and T-cells (Flajnik, 1996; Du Pasquier *et al.*, 2000; Courtet, Flajnik & Du Pasquier, 2001). Nevertheless, information on adaptive immunity in the skin of anurans, such as mucosal antibodies, is sparse. In other poikilothermic vertebrates such as teleost fishes, presence of antibodies in mucosal layers of the skin has clearly been demonstrated (Lobb & Clem, 1981; Hamuro *et al.*, 2007). In teleosts, immunity against a skin-dwelling parasite, the protozoan *Ichthyophthirius multifiliis*, is mediated by mucosal antibodies (Woo, 1997) and can be induced by immunization with parasite antigens (Wang *et al.*, 2002). Anurans likely have mucosal antibodies, but to our knowledge these are not yet described. Assuming that Woodhams

et al. will continue their interesting work on mucosal immunity in anurans, it is suggested to include experiments on potential adaptive immunity in the skin. A first informative trial could be to test if animals that have survived a chytridiomycosis infection are less susceptible to re-infection.

In conclusion, Woodhams *et al.* (2007) provide valuable information on the relevance of antimicrobial peptides in the skin of anurans. The implication that innate rather than adaptive immunity might establish resistance against fungal infections is extremely interesting, and awaits further investigation in these and other species. For conservation issues, it will be interesting to see whether declines in host populations might be linked to decreased genetic diversity in innate immune genes, in addition to genes of the adaptive immune system, such as the MHC (Acevedo-Whitehouse & Cunningham, 2006). More generally, in times of global change, declining host populations might enter a vicious circle of decreased immuno-genetic diversity in combination with emerging infectious diseases, a process that might also be relevant for other declining species, such as freshwater eels (Sures & Knopf, 2004). Defining the conditions under which species survive the threat of emerging pathogens will be one of the major joint challenges for ecological immunology and conservation biology.

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